Serotypes and virulence profiles of Shiga toxin-producing Escherichia coli strains isolated during 2017 from human infections in Switzerland

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Serotypes and virulence profiles of Shiga toxin-producing *Escherichia coli* strains isolated during 2017 from human infections in Switzerland

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Abstract

Since 2015, the Swiss Federal Office of Public Health registered an increase of notifications of STEC, probably due to the adoption of culture independent *stx* screening tests in diagnostic laboratories. This study aimed to identify the serotypes and virulence genes of 120 STEC isolated from human clinical *stx* positive specimens during 2017 in order to estimate any changes in serotype distribution and toxin profiles of STEC compared to the time span 2010–2014. Culturing of STEC from stool samples was achieved using the streak plate technique on MacConkey agar. We performed O and H serotyping by PCR and by micro array. Virulence genes were identified and subtyped using molecular methods, including *stx1* and *stx2* subtypes, and the intimin encoding gene, *eae*. STEC were recovered from 27.5% of the *stx* positive samples. STEC O157:H7 accounted for 7.5% of all isolates, and STEC O80:H2, O91:H10/H14/H21, O103:H2/H11, and O26:H11 accounted for 36.9% of the non-O157 strains. Forty-five isolates with *stx1* variants, 47 with *stx2* variants and 28 isolates with both *stx1* and *stx2* variants were identified. Forty (33.3% of all isolates) carried the subtypes associated with high pathogenic potential, *stx2a*, *stx2c*, or *stx2d*. The *eae* gene for intimin was detected in 54 strains (45% of all strains). Compared to 2010–2014, our data show that the proportion of the so called "top five" serogroups, STEC O26, O111, O103, and O157 declined from 53.7% to 28.3% in 2017. The proportion of isolates with *stx2a*, *stx2c*, or *stx2d* decreased from 50.5% to 33.3%. We also observed an increase of STEC harbouring the low pathogenic subtypes *stx2b* and *stx2e* from 12.6% to 29.2%, and of *eae* negative STEC from 29.5% in 2010–2014 to 55% in 2017. Simultaneously, there was a sharp increase of the patients' median age from 24 years to 46.5 years. Clinical manifestations in the patients included abdominal pain without diarrhea (22.3%), diarrhea (77.7%), and the haemolytic-uremic syndrome (HUS) (7.4%). Our data show that a greater number and a wider range of

STEC serotypes are detected by culture-independent testing, with implications for public health services.

Keywords

STEC, human, multiplex PCR, culture, serotypes

1. Introduction

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) are etiological agents of outbreaks and of sporadic cases of human gastrointestinal illnesses which may include non-bloody or bloody diarrhea, haemorrhagic colitis (HC), and the haemolytic uremic syndrome (HUS) (Karch et al., 2005). STEC are characterized by the production of one or more Stx, which consist of two groups designated Stx1 (consisting of the three variants Stx1a, Stx1c and Stx1d) and Stx2 (composed of seven distinct variants Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g). STEC associated with severe disease tend to feature variants Stx2a, Stx2c and Stx2d, whereas STEC producing Stx2b and Stx2e are linked to mild clinical symptoms or asymptomatic fecal carriage (Stephan and Hoelzle, 2000; Friedrich et al., 2002; Fuller et al., 2011). Virulence may further be increased by the presence of intimin, the product of the *eae* gene, which mediates attaching and effacing lesions on gastrointestinal epithelial cells (Kaper et al., 2004). STEC belonging to the serogroups O157, O26, O103, O111, and O145 constitute the so called "top five" serogroups of human pathogenic STEC in the EU and Switzerland, and are considered, together with a few others such as O91 and O113, important serogroups in public health (EFSA, 2017).

Human infection with STEC is a notifiable disease in Switzerland. Notification of confirmed cases to the federal office for public health (FOPH) is based on the isolation of STEC from faeces, or on the detection of *stx1* and/or *stx2* in faeces or from a clinical isolate of *E. coli*. The number of notifications of STEC in Switzerland has been increasing since 2015, possibly due to the introduction of PCR based, increasingly sensitive *stx* screening tests in laboratory testing practices. However, such culture-independent testing (CIDT) generally does not yield an isolate, and positive results are not always culture confirmed. From the public health point of view, the advantages of rapid and broad range pathogen detection are therefore challenged by possible loss of strain subtyping with consequent disruption of monitoring trends in

serotype or Stx distribution (Cronquist et al., 2012). Recognition of the trends in serotypes and toxin profiles of STEC is however of great importance in order to estimate their potential for causing disease and to anticipate epidemiological changes. The aim of this study was to gain epidemiological and serotyping information on STEC isolated during 2017. Therefore, from May to December 2017, the FOPH requested all diagnostic laboratories to forward clinical materials that tested positive for *stx* by CIDT to the Swiss National Reference Centre for Enteropathogenic Bacteria and *Listeria* (NENT) for culture and further strain characterization. The isolates were analysed with regard to their serotypes, *stx* subtypes and presence of the *eae* gene. The results were compared with earlier data from Switzerland investigated over the 5-year period 2010-2014 (Fierz et al., 2017).

2. Material and Methods

2.1. Sample collection

Human stool samples that tested positive for *stx* using multiplex molecular methods were submitted to the NENT from May to December 2017 from clinical diagnostic laboratories distributed nationwide.

From a total of 457 submitted specimens, 436 were included for analysis after the elimination of repeat specimens (i.e., specimens obtained from the same patient). Of the 436 patients, data of age and gender were known for 431 individuals. Thereof, 240 (55.7%) were from female and 191 (44.3 %) were from male patients. The median age was 46.5 years (range 0–99 years). Forty-six (10.7%) were isolated from patients ≤ 5 years of age.

2.2. Strain isolation

Specimens were cultured on MacConkey agar using the streak plate technique. From each plate, six individual colonies, if possible of different morphology, were picked und subcultured on sheep blood agar (Difco™ Columbia Blood Agar Base EH; Becton Dickinson AG, Allschwil, Switzerland). Isolates that were confirmed to possess *stx (stx1 and/or stx2)* by real-time PCR (LightCycler R 2.0 Instrument, Roche Diagnostics Corporation, Indianapolis, IN, USA) (EURL, 2013a) were selected for further analysis. From plates yielding more than one *stx* positive colony, one isolate was randomly chosen for subsequent characterization. Proportions of STEC in stool samples were defined as the numbers of *stx* positive colonies among six *E. coli* colonies.

2.3. Serotyping

Strains were examined by PCR for the presence of genes associated with 14 selected serogroups including the top-five serogroups, namely O26, O45, O55, O80, O91, O103, O104, O111, O113, O121, O128, O145, O146, and O157 (Perelle et al., 2004; EURL, 2013a; EURL, 2014; Soysal et al., 2016). Strains were tested for the presence of flagellar genes related to H2, H4, H7, H8, H10, H11, H19, H21, H25, and H28 (Mora et al., 2012; EURL, 2013b; Beutin et al., 2015; Alonso et al., 2017). Strains belonging to other O groups and H types were serotyped using the Alere™ *E. coli* SeroGeno typing AS-1 kit (Alere Technologies, Jena, Germany).

2.4. Virulence markers

The identification of *stx1* subtypes (*stx1a, stx1c*, *stx1d*) and *stx2* subtypes (*stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, *stx2g*) was carried out by conventional PCR amplification (Scheutz et al., 2012). Screening of the strains for *eae* was performed by real-time PCR according to the guidelines of the European Union Reference laboratory (EURL, 2013a).

3. Results

3.1. Recovery rate

Out of a total of 436 human fecal specimens that tested positive by multiplex PCR for the presence of *stx*, 120 samples yielded an STEC isolate for further characterisation, amounting to a recovery rate of 27.5%.

Categorising the 120 samples into those with high numbers of *stx* positive colonies (five or six positive colonies per sample), those with intermediate numbers (three or four positive colonies) and those with low numbers (one or two colonies), resulted in 46 (38.3%) stool samples with a high proportion of STEC colonies, 35 (29.2%) with an intermediate proportion, and 39 (32.5%) stool samples with a low proportion of STEC, respectively Strains exhibiting high or low colony numbers are listed in Table 1.

3.2. Serological diversity

Twenty-five different O-serogroups were identified among the 120 STEC isolates, in addition to 17 O-non-typeable (Ont) serogroups, and 4 ambiguous results. Eighteen different H-types were determined, including two non-typeable H-types and two ambiguous results. An overview of the serotypes is given in Table 1.

Among the 120 isolates, 9 (7.5%) were O157:H7, and 111 (92.5%) were non-O157 STEC strains. Together with STEC O157, the top five serogroups were represented by STEC O103 (n=11), O145 (n=6), and O26 (n=8), amounting to 28.3% of the isolates. No STEC O111 were detected. Isolates belonging to O80:H2 (n=11), and O91:H10/H14/H21 (n=11) accounted each for 9.2% of the isolates, respectively. Other serotypes included O174:H2/H8/H21 (n=7; 5.8%), and O146:H21 (n=6; 5%) (Table 1). Other serotypes were represented by four or less STEC isolates (Table 1).

149

150 **3.3. Distribution of virulence genes among the serotypes**

151 Of the 120 STEC strains, 45 (37.5%) carried *stx1* genes only: *stx1a* (n = 35), *stx1c* (n = 9)
152 and *stx1d* (n=1). Forty-seven strains (39.1%) carried *stx2* genes only: *stx2a* (n = 14), *stx2b* (n
153 = 14), *stx2c* (n = 5), *stx2d* (n = 12), and *stx2e* (n = 2). Twenty-eight (23.3%) harboured
154 combinations of *stx1* and *stx2* genes. Forty (33.3% of all isolates) carried the subtypes
155 associated with high pathogenic potential, *stx2a*, *stx2c*, or *stx2d* (Table 1). The
156 majority thereof (n = 25/62.5% of the 40 strains) were associated with O80:H2 (n=11),
157 O157:H7 (n = 9) and O145:H28 (n = 5).
158 Thirty-five (29.2%) isolates harboured the low pathogenic subtypes *stx2b* and *stx2e* and were
159 mainly associated to the serogroup O146 and Ont serogroups.
160 The *eae* gene encoding intimin was detected in 54 strains (45% of all strains). Thereof, 31
161 (57.4% of the *eae* positive strains) were associated with *stx2a*, *stx2c*, or *stx2d*, and one (4.3%)
162 with *stx2b*. The remaining 22 (40.7%) of the *eae* positive isolates carried *stx1a* alone (Table
163 1).
164 The majority (25 strains, 80.6%) of the *eae* positive strains harbouring *stx2* subtypes
165 belonged to STEC O80:H2, O145:H28, and O157:H7. By contrast, of the 66 (55% of all
166 strains) that tested negative for *eae*, nine (13.6% of all *eae* negative strains) harboured *stx2a*,
167 *stx2c*, or *stx2d*, while 34 (51.5%) were associated with *stx2b* or *stx2e*. The remaining 23
168 (34.8%) carried *stx1a stx1c* or *stx1d* alone (Table 1).
169 The distribution of serogroups and genotypes compared to earlier data from 2010-2014 (Fierz
170 et al., 2017) is illustrated in Figure 1.
171

3.4. Relationship between STEC type, age of patients, clinical symptoms, and proportions of *stx* positive *E. coli* in stool samples

Patients were classified into four groups, according to their age at the time of sampling. Age group 1 consisted of infants and children ≤ 5 years of age (n=14), group 2 contained children and young adults between 6 and 17 years (n=16). Group 3 consisted of adult patients between 18 and 60 years (n=56), and group 4 of patients >60 years (n=33). For one patient, the age was unknown (Table 1). Clinical data were provided for 94 (78.3%) of the patients. Abdominal pain without diarrhea (AP) was reported for 21 (22.3%) of the patients. The majority (77.7%) suffered from diarrhea (D). HUS was present in 7 (7.4%) of the patients, six thereof with D, and one with AP. Two further patients (2.1%) had presumptive HUS with acute kidney failure (AKF). Nineteen (20.2%) patients were hospitalized (two patients with AP only and 17 patients with D).

The distribution of STEC serotypes and of *stx2* and *eae* genes among the patients' age groups is listed in Table 1. STEC belonging to the top five serogroups were found in 71.2% of the STEC infected children from age group 1 and in 56.3% from age group 2. Infections due to the top five STEC serotypes were less frequent among age group 3 (16.1% of the patients), and age group 4 (18.2% of the patients). By contrast, of the two most prevalent serogroups from this study, STEC O80 was isolated more frequently from patients in age group 4 (15.2% of the patients) than from patients in groups 1 (7.1%), group 2 (6.3%) and group 3 (7.1%), respectively. STEC O91 was detected only among isolates from patients of age groups 3 and 4, accounting for 16.1% and 6% of the infections, respectively. STEC harbouring *stx2a*/*stx2d*/*stx2c* were frequent among patients from age groups 1, 2 and 4 (50%, 43.8%, and 42.4% respectively), and least frequent among patients from age group 3 (19.6%). STEC containing *stx2b*/*stx2e* was more frequent among patients from age groups 3 and 4 (33.9% and 33.3%, respectively), compared to patients from age groups 1 and 2 (7.1% and 25%,

198 respectively). Similarly, *eae* positive STEC were observed at higher rates among patients
199 from groups 1 and 2 (85.7% and 62.5%, respectively) than among those from groups 3 and 4
200 (30.4% and 42.2%, respectively). By contrast, *eae* negative STEC were remarkably less
201 frequent in isolates from patients of age groups 1 and 2 (14.3% and 37.5%, respectively) than
202 in those from group 3 and 4 (69.6% and 57.6%, respectively). The distribution of STEC
203 serogroups and of *stx2* and *eae* genes among the patients' age groups is illustrated in Figure
204 2A and B, respectively.
205 STEC serotypes and virulence genes associated with patients with abdominal pain only,
206 diarrhea, HUS, and with patients that were hospitalised are listed in Table 1. STEC belonging
207 to the top five serogroups were found in 14.3% of 21 patients with AP, in 30.1 % of 73
208 patients with D and in 42.9% of 7 patients with HUS (Table 1). The top five serogroups were
209 furthermore associated with 31.6% of 19 hospitalised patients. STEC O80 was isolated from
210 4.8% of patients with AP and 12.3% of patients with D. Moreover, STEC O80:H2 was
211 associated with one case of HUS, two cases of AKF and isolated from 21% of hospitalised
212 patients (Table 1). STEC O91 was detected at similar rates among patients with AP and D
213 (14.3% and 9.6%, respectively), in one HUS case and in 15.8% of hospitalised patients.
214 STEC harbouring *stx2a*/*stx2d*/*stx2c* were less frequent among patients with AP (14.3%), than
215 among isolates from patients with D (41%) and HUS patients (100%), and were found in
216 68.4% of hospitalised patients. Similarly, *eae* positive STEC were observed at a lower rate in
217 patients with AP (23.8%) than among patients with D (47.9%) or HUS (85.7%), and
218 hospitalised patients (63.2%) (Table 1). By contrast, STEC carrying *stx2b*/*stx2e* were more
219 frequent among patients with AP (57.2%) than among patients with D (21.9%), and absent
220 among HUS patients.

Finally, *eae* negative STEC were accountable for 76.2% of patients with AP only, and for 52.1% and 14.3% of patients with D and HUS, respectively. Furthermore, *eae* negative STEC were recovered from 36.8% of hospitalised patients.

The distribution of STEC serogroups and of *stx2* variants and *eae* genes among patients with AP, D, HUS, and hospitalized patients is illustrated in Figure 2C and D, respectively.

The proportions of STEC among *E. coli* isolated from the patients' stool samples varied according to serotypes and virulence genes. The majority of the STEC O145, STEC O157, and STEC O91 isolates (66.7%, 55.6%, and 54.5%, respectively) were found in high numbers in the stool samples, whereas high proportions were less frequent for serogroups O26 (50%) and O103 (36.4%), and remarkably less (28.1%), for isolates belonging to serogroups other than the top five, O80 or O91 (Figure 2E). STEC isolates harbouring *stx2a*/ *stx2c*/ *stx2d* were observed more frequently in higher proportions than those harbouring *stx2b*/*stx2e* (50% and 34.3%, respectively). Similarly, *eae* positive STEC were found more frequently in high proportions among *E. coli* from patients' stool samples than *eae* negative STEC (44.4% and 33.3%, respectively, Figure 2F).

Among patients with AP, the proportion of STEC in stool was high for 33.3% and low for 47.6%. Among patients with D and HUS, proportions were high for 37% and 42.9%, respectively, and low for 32.9% and 28.6% (Table 1).

4. Discussion

Since 2015, the Swiss FOPH has registered an increase of notifications of STEC related infections compared to previous years, with the increasing use of *stx* screening tests driving this trend (Hächler and Stephan, 2015). In order to estimate any changes in serotype

distribution and toxin profiles of STEC, this study aimed to identify the serotypes and virulence genes of 120 STEC isolated from human clinical specimens during 2017.

The five most common serogroups were O157, O103, O26, O91 and O80, with *E. coli* O157:H7 accounting for 7.5% of the STEC strains. By comparison, during 2000–2009, 30.6% of the STEC strains isolated from humans in Switzerland were *E. coli* O157:H7, and during 2010–2014 this rate further decreased to 19% (Käppeli et al., 2011; Fierz et al., 2017). Thus, as reported for other countries in the EU, the proportion of STEC O157 isolated from humans continues to decrease in Switzerland (EFSA, 2017). Similarly, the proportion STEC O145 which belongs to the top five serogroups in Europe (EFSA, 2017), decreased from 12.6% during 2010–2014 to 5.4% in 2017 (Fierz et al., 2017). By contrast, with regard to the serogroups that do not belong to the top five, we observed an increase of STEC O80 from 6.3% to 9.2% and of STEC O91 from 3.2% to 9.2%, compared to 2010–2014 (Fierz et al., 2017). In particular, the increase of STEC O80:H2 is noteworthy. This hypervirulent, multidrug resistant serotype harbours genetic characteristics of a hybrid STEC/ extraintestinal pathogenic *E. coli* (ExPEC) pathotype and has recently emerged in France and Switzerland associated with severe disease including bacteremia and HUS (Soysal et al., 2016; Fierz et al., 2017; Nüesch-Inderbinen et al., 2018).

Compared to the study period 2010-2014, we also observed a remarkable decrease in the percentage of STEC harbouring *stx2* variants that are associated with severe disease (i.e., *stx2a*, *stx2c*, *stx2d*) from 50.5% to 33.3%, and of *eae* positive STEC from 70.5% to 45%. Simultaneously, we noted a sharp increase from 12.6% to 29.2%, of STEC associated with *stx2b* and *stx2e*, variants that are linked to mild clinical symptoms and asymptomatic carriage (Stephan and Hoelzle, 2000; Fuller et al., 2011), and to *eae* negative STEC from 29.5% to 55%. Correlating to this development, the patients' age median increased from 24 years to

46.5 years, and the proportion of patients ≤ 5 years dropped from 43.2% in 2010–2014 to 10.7% in 2017.

The present study analysed STEC cultured stool samples that were positive for *stx* by PCR screening. The relatively low success rate of 27% for isolation of STEC by culture compared with other studies 72%-96.5% (Friedrich et al., 2002; Tunsjø et al., 2015) may be explained by the loss of *stx* genes over time due to transport conditions or time delay to culture in the reference laboratory. A further explanation is that the concentration of STEC in some of the stool samples may have been too low to allow detection of isolates. Alternatively, the low recovery may have been caused by the use of PCR to directly target *stx* genes in faeces. Free *stx*-carrying bacteriophages have been detected at a prevalence of 62% in fecal samples of healthy people and may thus be responsible for some of the *stx* positive stool samples (Martinez-Castillo et al., 2013; Urdahl et al., 2013). Therefore, caution should be employed when diagnosing disease on the basis of *stx* positive results obtained directly from faecal samples.

A definite attribution of the STEC isolates from this study to disease was precluded by the lack of sufficient information on co-detection of pathogens other than STEC by multiplex PCR based CIDT. Overall, it cannot be excluded that a certain number of the isolated STEC were not the actual etiological agent of disease. Indeed, ten (8.3%) of the STEC strains identified in this study belong to serotypes detected previously in faecal samples of healthy humans including O8:H25, O128:H10, O146:H21, O146:H28, and O174:H8 (Stephan and Untermann, 1999; Stephan and Schumacher, 2001; Urdahl et al., 2013).

Our data are supportive of previous studies that established an association of *eae* negative STEC with severe clinical symptoms including HUS (Beutin et al., 2004). However, further studies are needed in order to correlate the presence of infrequently detected STEC serotypes

and *stx2b*/*stx2e* harbouring STEC with the severity of disease and, ultimately, to distinguish public health relevant infections from the non-relevant.

Conclusively, in spite of the increased notification of STEC during 2017, there was no evidence for an outbreak situation. Our data are suggestive of an ongoing trend towards a wider spectrum of serologically different STEC types most likely due to the introduction of PCR based, increasingly sensitive *stx* screening tests in laboratory testing practices. This study emphasises the importance of combining molecular methods of detection with classical culture techniques to enable the detection of emerging STEC serotypes or outbreak situations. Moreover, recognition of the trends in serotypes and toxin profiles of STEC is of great importance in order to estimate epidemiological changes.

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Conflicts of interest

None to declare.

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414 **Figure legends**

415 Figure 1: Comparative distribution of serogroups and genotypes of STEC isolated during
416 2010-2014 and in 2017 from humans.
417 A: Percentage of the "top five" STEC serogroups and other selected STEC serogroups
418 isolated during 2010-2014 and in 2017.
419 B: Percentage of STEC harbouring *stx1* and *stx2* subtypes, and percentage of *eae* positive and
420 *eae* negative STEC isolated during 2010-2014 and in 2017.
421 Figure 2: Relationship between STEC type, patients' age, clinical symptoms, and proportions
422 of STEC types among *E. coli* in stool samples.
423 A: Percentage of patients by age group infected with isolates belonging to the top five STEC
424 serogroups (O26, O103, O111, O145, or O157), STEC O80, STEC O91, or other STEC
425 serogroups.
426 B: Percentage of patients by age group infected with STEC harbouring *stx2* virulence genes
427 associated with high pathogenicity (*stx2a*/*stx2c*/*stx2d*), *stx2* genes associated with milder
428 symptoms (*stx2b*/*stx2e*), *eae* positive (*eae* +) STEC, and *eae* negative (*eae* -) STEC.
429 C: Distribution of STEC serogroups isolated among patients with abdominal pain only (AP),
430 diarrhea (D) or the hemorrhagic uremic syndrome (HUS), and from hospitalised patients (H).
431 D: Distribution of STEC harbouring *stx2* virulence genes, and percent *eae* + and *eae* - STEC
432 among patients with AP, D or HUS and among hospitalised patients.
433 E: Percentage of selected STEC serogroups present in high or low proportions among *E. coli*
434 isolated from human stool samples. Stool samples with five or six *stx* positive colonies out of
435 six *E. coli* colonies were classified as high. Samples with one or two STEC per six *E. coli*
436 colonies were defined as low proportion.

F: Percentage of STEC harbouring *stx2* subtypes, and percentage of *eae* positive and *eae* negative STEC present in high or low proportions among *E. coli* isolated from human stool samples.